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GB 1461319
GB 1424823
GB 1401227
GB 1381514
GB 1335483
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GB 1218745
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(54) Testing apparatus for analysis of liquid samples

(57) In apparatus for analysis of liquids of the type which uses disposable discrete analyte dedicated substrates incorrect results arising if the substrate is inoperable through being defective or dedicated to a different analyte from that being measured, are avoided by combining the response measuring means used to monitor the response of the substrates to applied analyte samples with testing means adapted to distinguish defective substrates or wrongly dedicated substrates and initiate their removal. Defective substrates may be detected by failure to react with analyte and a resulting absence of differential signal and wrongly dedicated substrates comprising electrodes may be detected by measuring electrode impedance.

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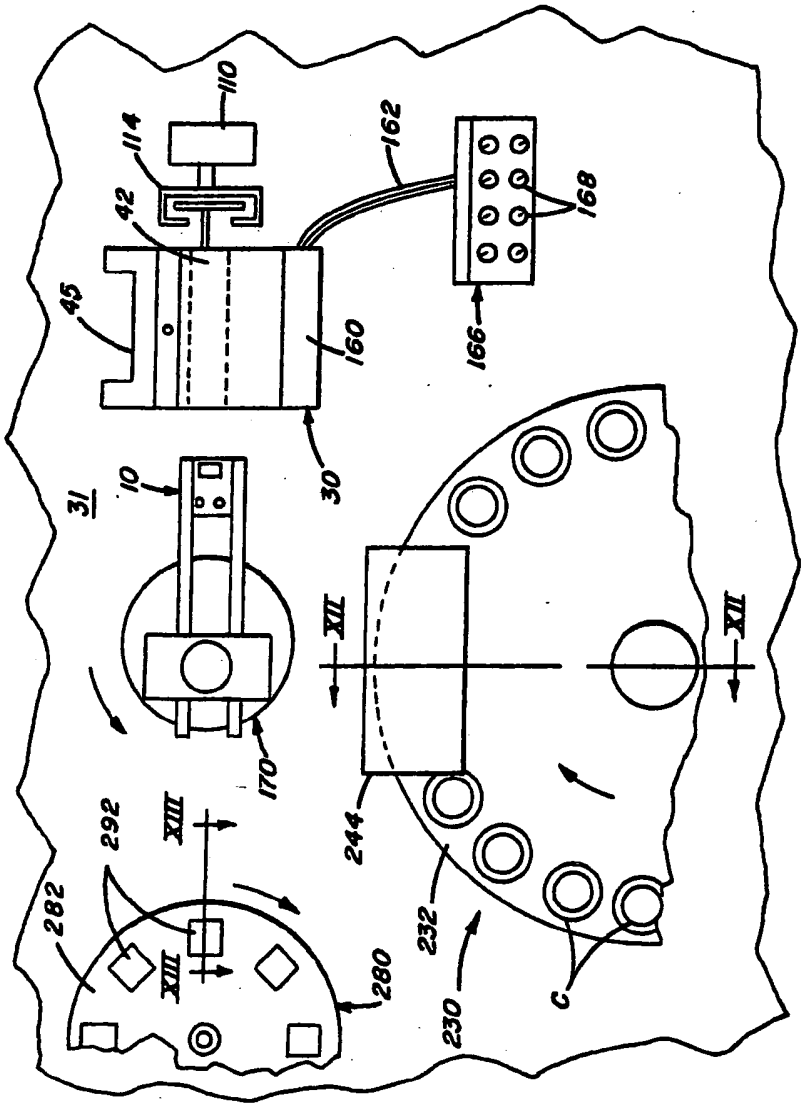

FIG. 1

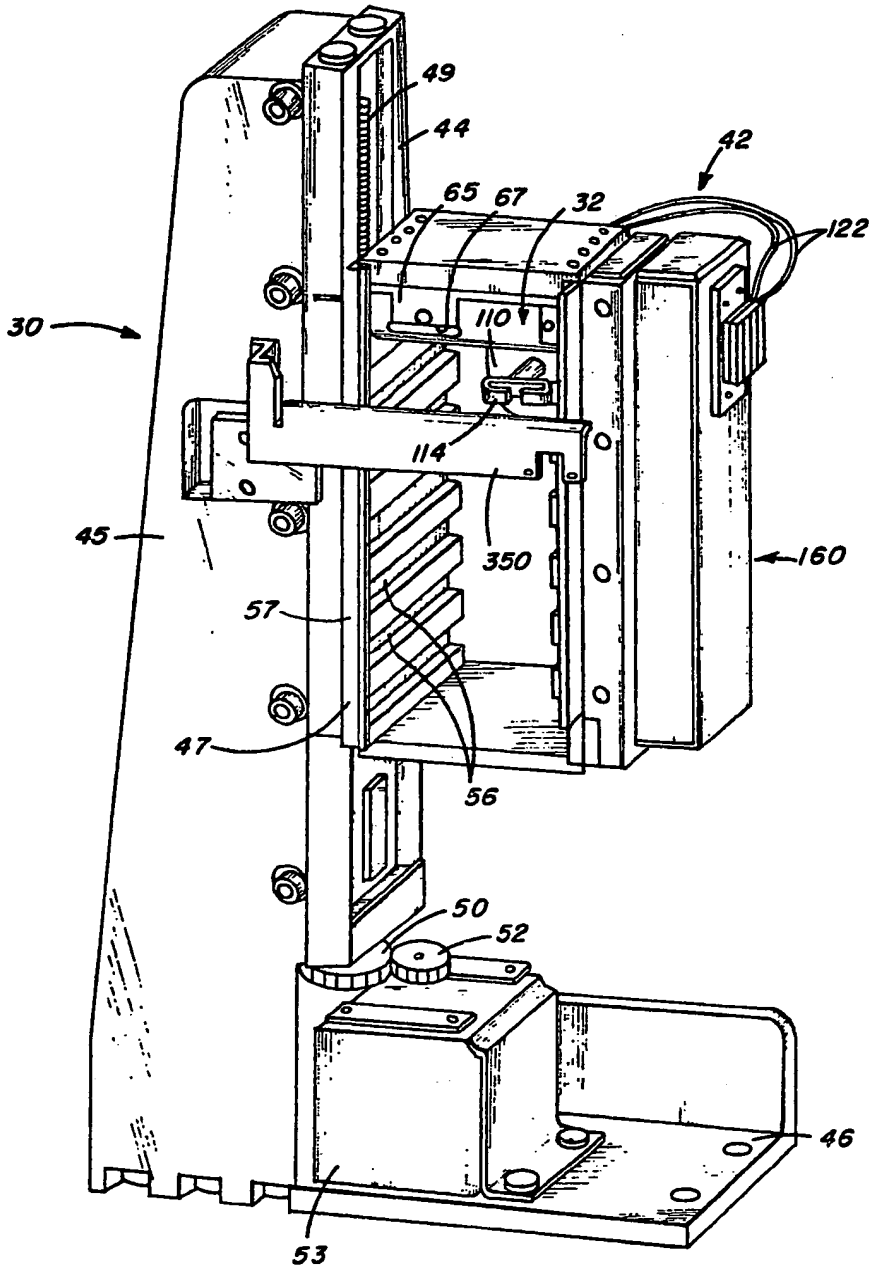

FIG. 2

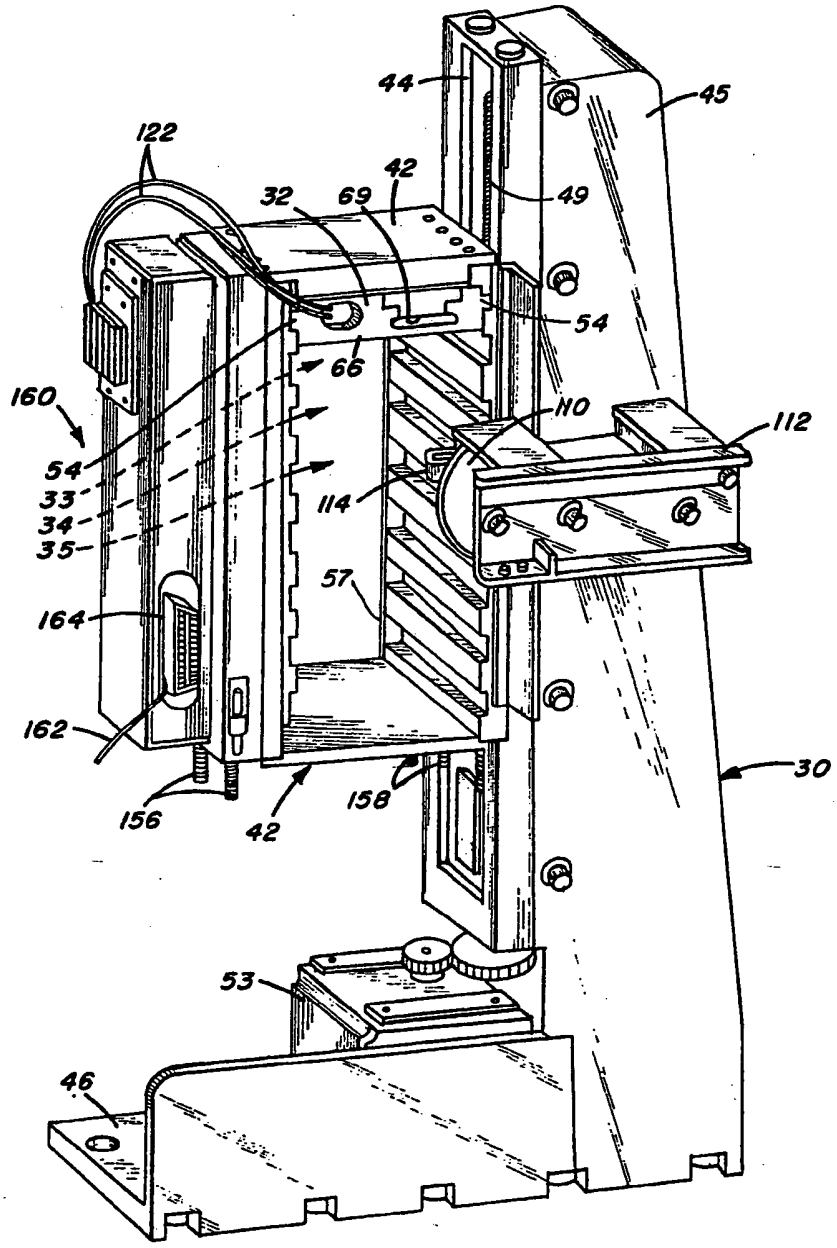

FIG. 3

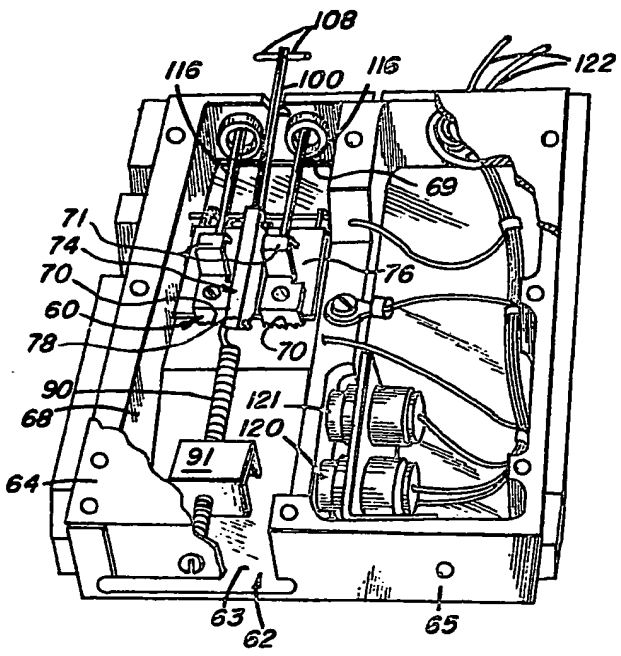

FIG. 4

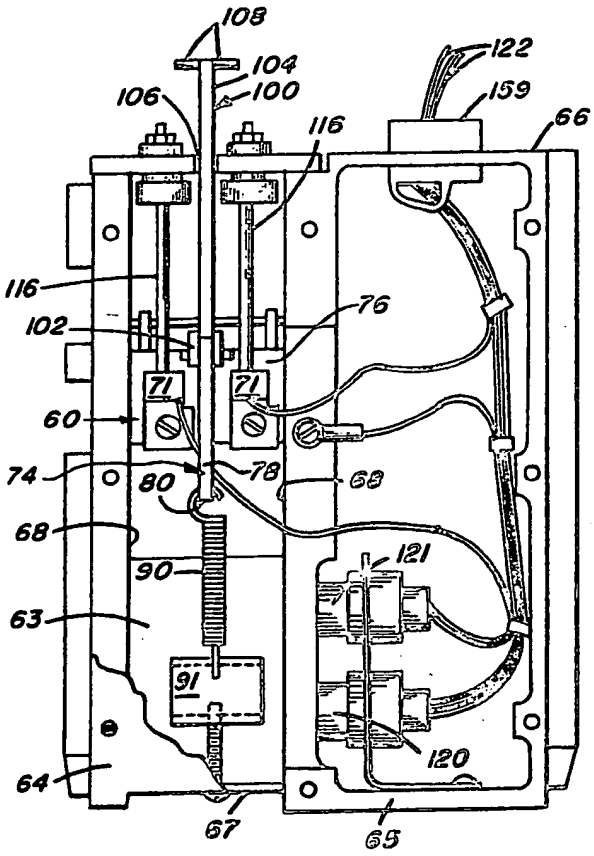

FIG. 5

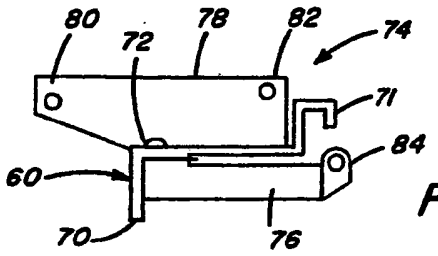

FIG. 6

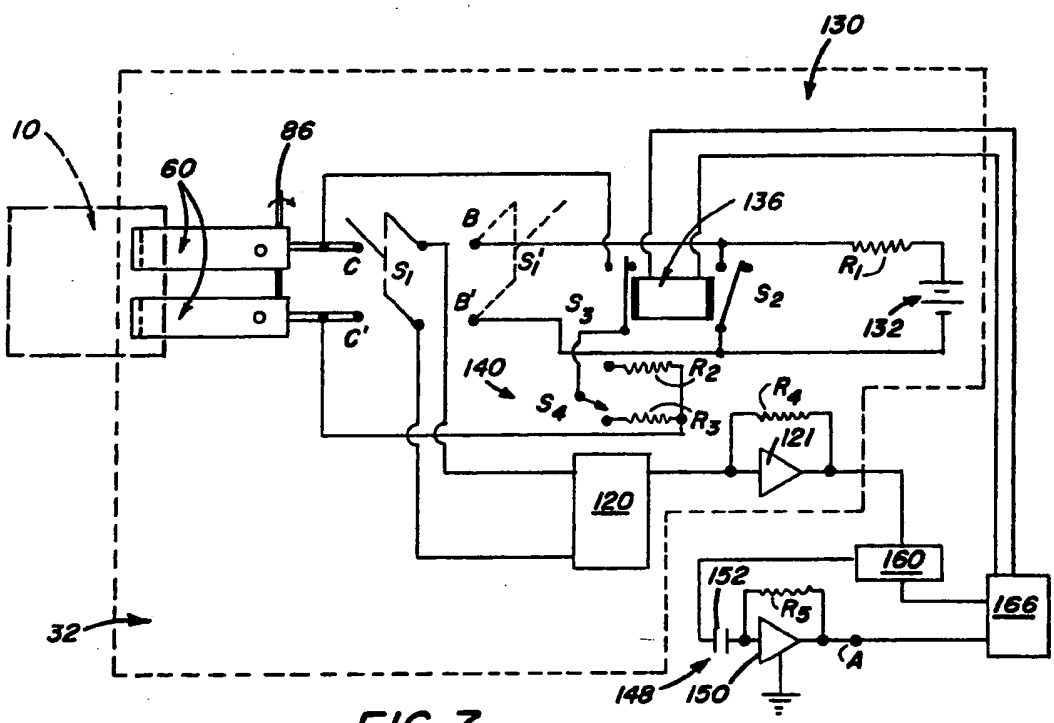

FIG. 7

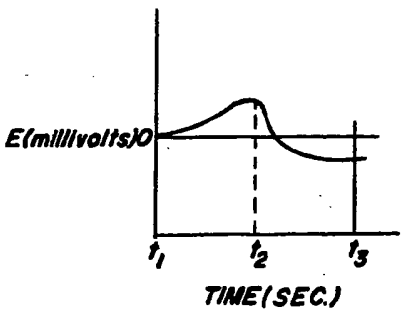

FIG. 8A

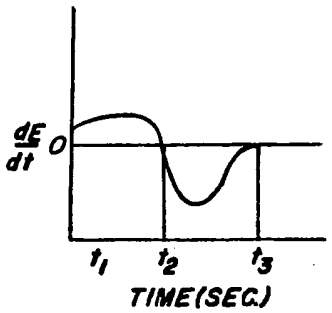

FIG. 8B

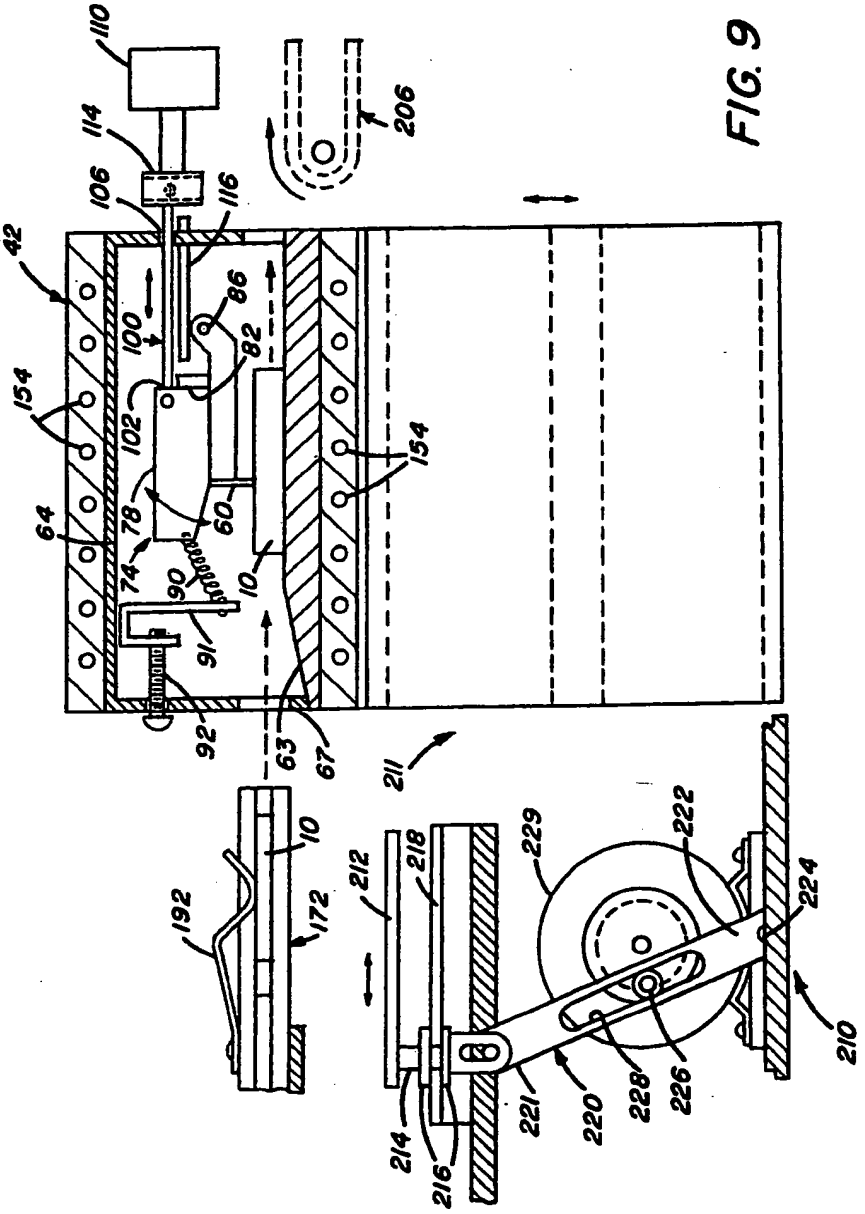

FIG. 9

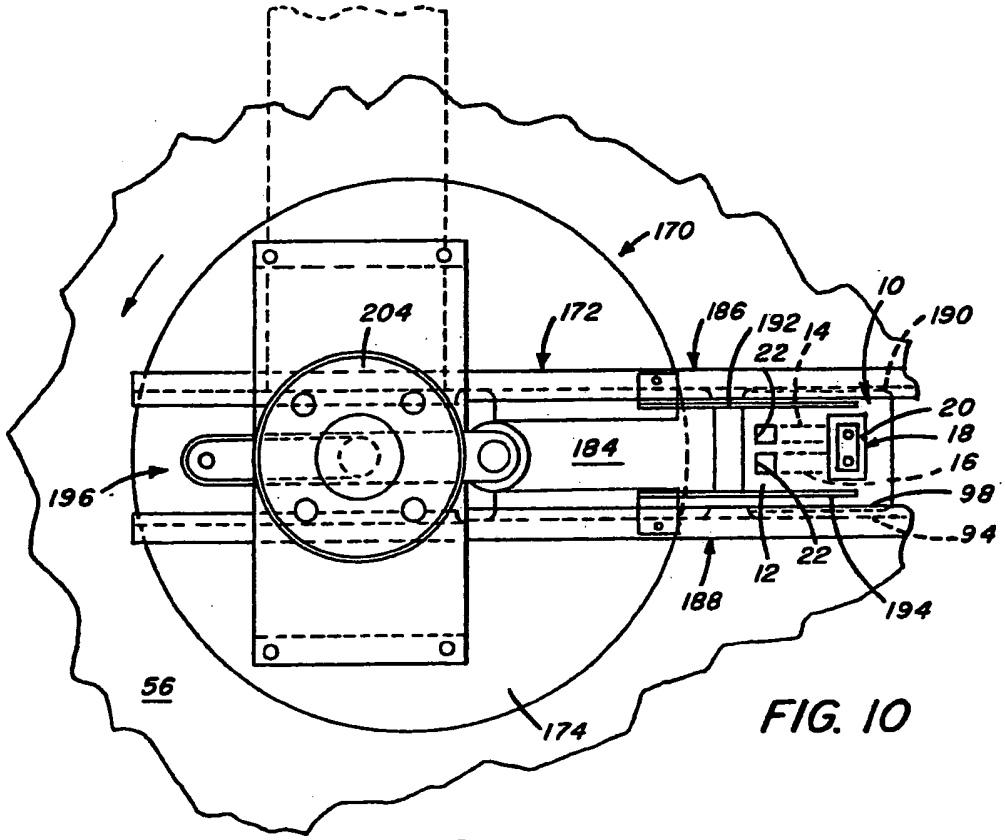

FIG. 10

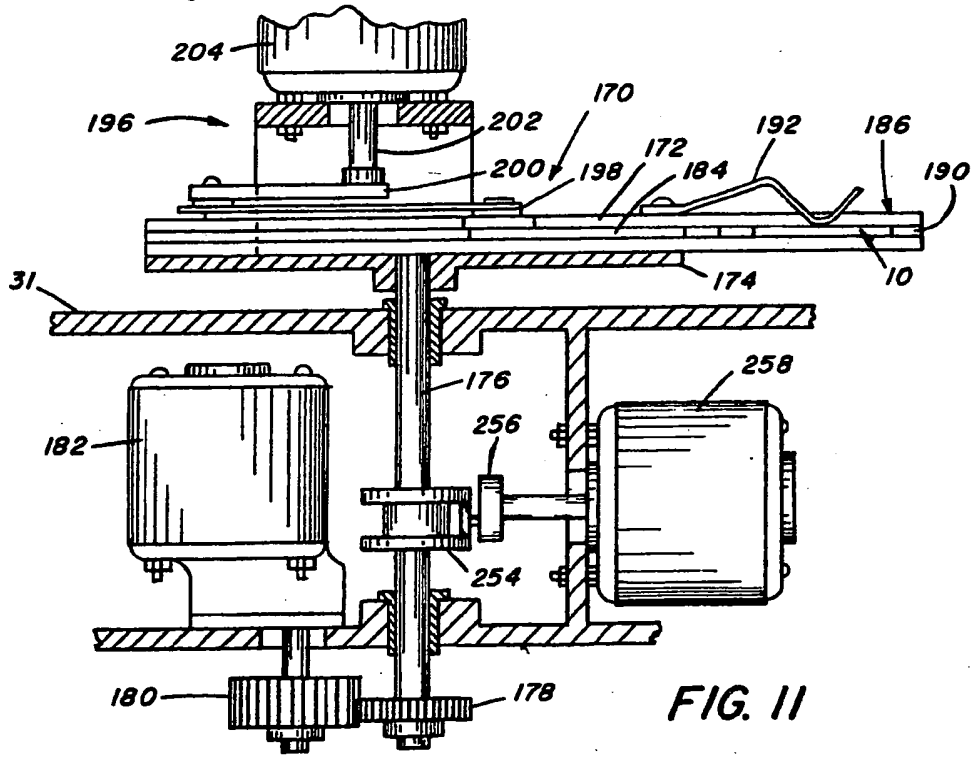

FIG. 11

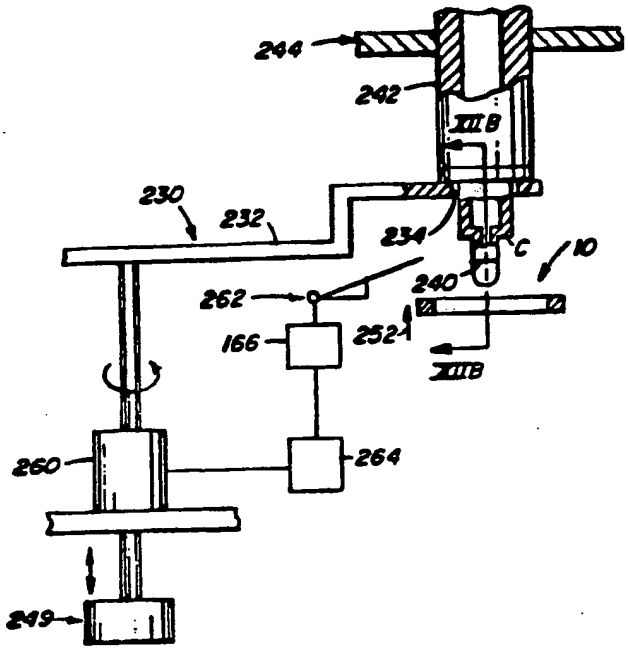

FIG. 12A

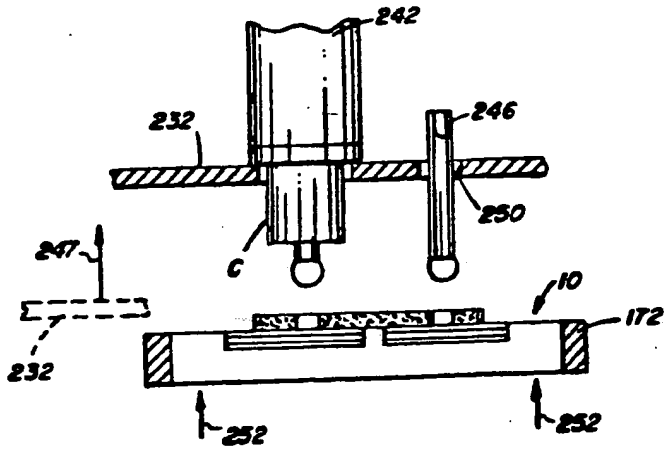

FIG. 12B

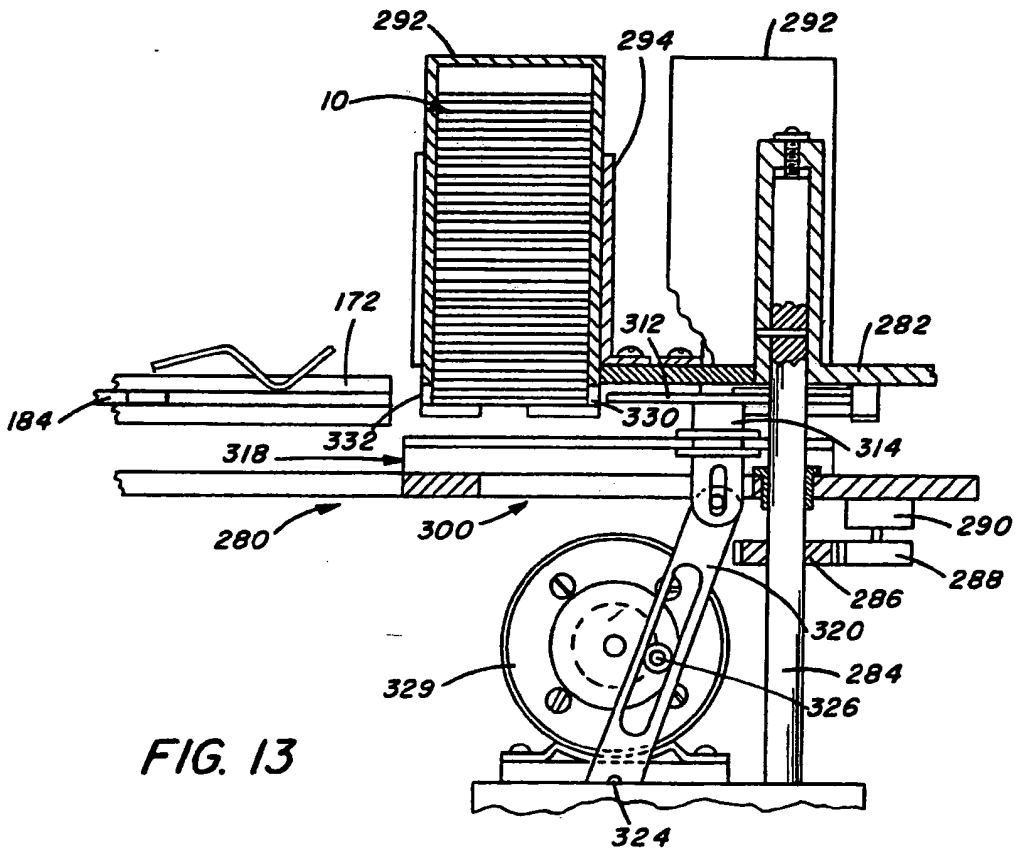

FIG. 13

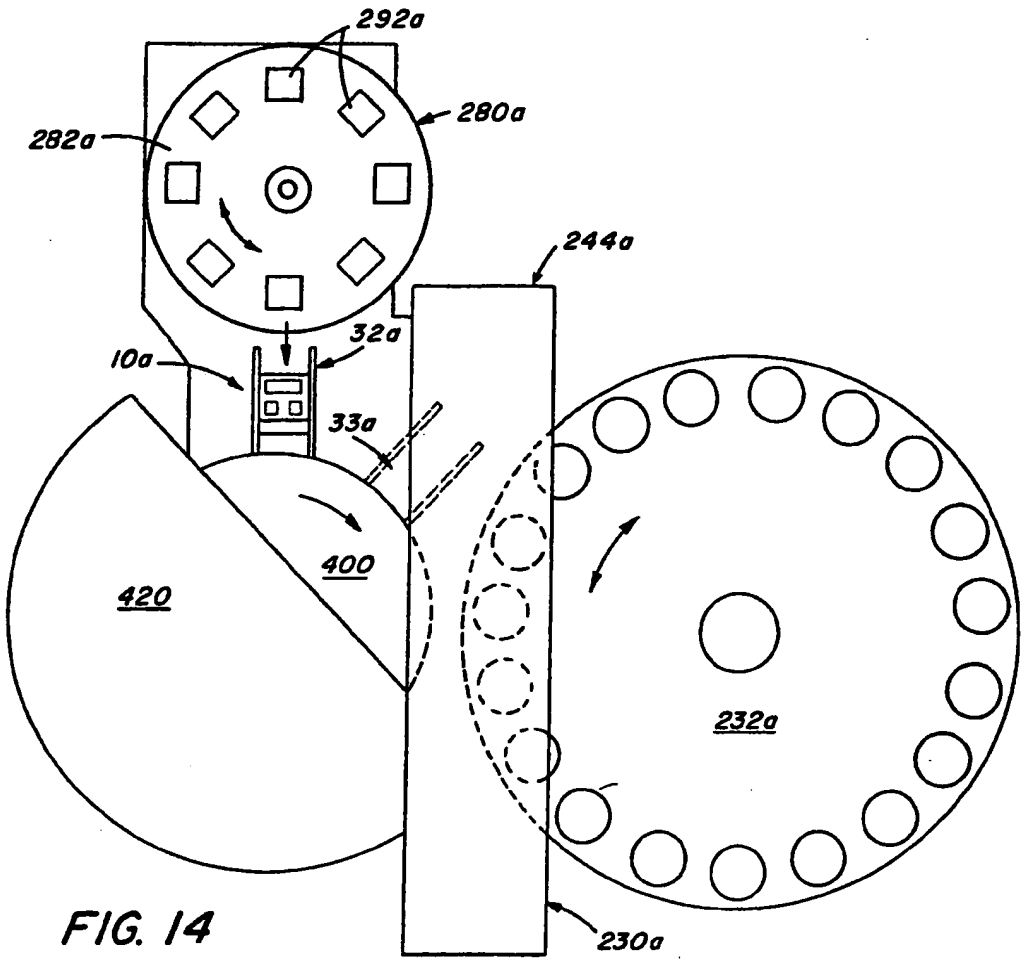

FIG. 14

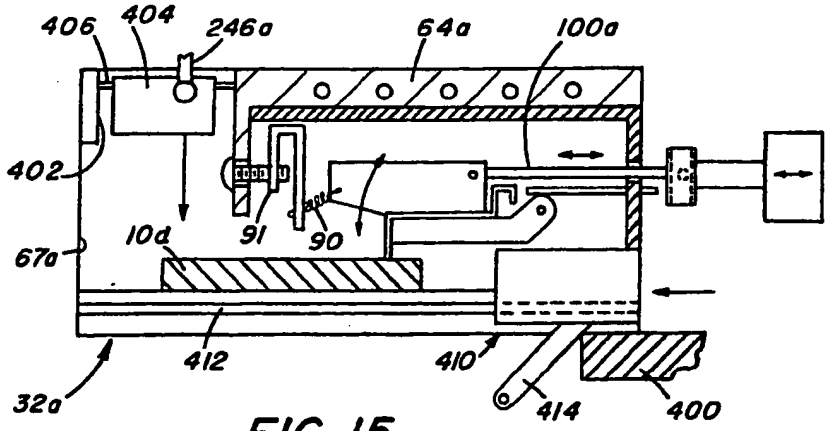

FIG. 15

SPECIFICATION

Apparatus for analysis of liquid samples

The present invention relates to apparatus for detecting the level of various analytes in liquids such as body fluids. Apparatus of this type is commonly known as a clinical analyzer.

Clinical analyzers have been developed to permit analysis of body fluids, such as blood serum, whereby physicians can both detect and measure the amount of various analytes whose presence or level may be used to assist diagnosis of body malfunction or disease. Such liquids, if added to appropriate reagents, which can be either in a liquid or in a dried, coated form generate a signal, for example in the form of a colour change or fluorescence. Detection of such radiation signals after a suitable incubation period is usually done in a single station as a final step. Generally such analysis is called radiometric detection. Alternatively, certain ionic blood components are detected electrically by a potentiometer using the potentials generated by ion selective electrodes, hereinafter ISE's. Included in such tests are the amounts of chloride, sodium and potassium ions. Whether the test is potentiometric or radiometric, it relies upon a substrate to generate a signal of the respective kinds described, in the presence of the test component of the biological liquid.

Recent innovations have provided ISE's in essentially planar, dried form, suitable for use in pairs in an analyzer. Examples are described in Research Disclosure Issue 161 September 1977, Item No. 16113 entitled "Ion-Selective Electrode" and U.S. Patent No. 4,053,381 "Device and Method for Determining Ionic Activity and Components of Liquid Drops". Although remarkable accuracy is achievable by such devices, it has been discovered that occasionally the paired electrodes are shorted together, and therefore use less for testing for ion activity. Ion selective electrodes, by their nature, are dedicated to the measurement of a single ion. A pair of ISE's designed for testing for chloride ions however could be assigned erroneously for testing for sodium ions for example giving faulty output. Such defects and errors can occur even with good quality control in manufacturing. When a defective or erroneous substrate is used, the problem is detected, if at all, in the analyzers of the prior art only when the read station is reached, necessitating that the whole test be repeated. If the wrong type of ISE substrate has been used, it is possible that the error might not be detected at all so that a false result is reported. In any event, the container from which the sample in question was drawn for analysis generally might no longer be available, in which case a new blood sample must be drawn from the patient to repeat the test with a new substrate.

Yet another problem which can exist in some solid electrodes is a differential rate of equilibrium arising out of a nonuniformity of coating thickness and chemistry. That is, with some electrodes it is not possible to predict that a steady-state equilibrium will be reached in all cases in x minutes, unless "x" is selected to be as long as the longest possible time. An arbitrary selection of a large value of "x" results in wasted throughput time compared to what would be available if individual equilibrium times were known.

Defective substrates can also occur in radiometric analyzers, which as noted above rely upon a single photometer or colorimeter to read each substrate at the end of a test. For example, insufficient or incorrect chemicals may have been added to form the substrate. Such a problem will be detected, if at all, only when the single read station, hereinafter called "single channel", is finally reached, necessitating the retrieval of another blood sample from the patient to repeat the test. As with ion selective electrodes, the substrates used in radiometric analysers are normally dedicated to a single analyte.

Single channel analyzers of the type described above inherently require the read station to be last, because any attempt to place the read station earlier in the sequence would cause all other test samples to be delayed for n times t minutes, where n is the number of substrates to be read and t is the duration of the incubation period. The through-put of the machine would be greatly delayed beyond that which is economically practical.

The analyzer disclosed in United States Patent No. 3,832,135 is an example of the radiometric analyzers described above. Although there are a plurality of radiometers in that analyzer, there is only one radiometer for each test, so that on a test-by-test basis, it provides only a single channel read-out.

In the *Centria* system of Union Carbide, described in *Clinical Chemistry*, Volume 21, No. 9, page 1305 (1975), through-put is increased by using three detection stations for radioactive immunoassays. This system fails to utilize such multiple stations to provide early detection of possibly erroneous substrates, in part because an intermediate process step (centrifuge separation) prevents such early detection. That is, after the sample is added to the reagent it is transferred into an intermediate incubator/separator (I/S) station, after which the I/S stations are manually moved to a reader module that contains the detection stations. Furthermore, there is not even any appreciation that such early detection is desirable or could avoid errors.

Scintillation counters for the analysis of radioactive samples have been developed with multichannel read-out, as shown for example in U.S. Patent No. 4,005,292. However, as

with the Centria system, no attempt has been made in such systems to utilize such multichannel read-outs to provide early detection of erroneous substrates.

According to the present invention there is provided apparatus for analysis of liquids of the type which accepts disposable discrete analyte dedicated substrates and monitors the response of the substrates to applied analyte samples characterised in that testing means associated with the response measuring means includes testing means adapted to discard substrates which are inoperable due to a defect or being dedicated to a different analyte from that being measured.

The problems of the prior art devices are solved by obtaining a record of the initial and subsequent signals generated by the substrate, and this in turn is achieved by transferring the substrates directly to the read stations after the sample is deposited, without passing the substrate through intermediate, non-reading stations as is common in prior art analyzers. The use of multichannels avoids the delay in processing that would occur in a continuously monitoring single channel analyzer.

More specifically, in accordance with one aspect of the invention, such an analyzer is provided comprising a plurality of stations, each of which is constructed to receive a substrate bearing a sample for analysis; individual sensing means at each of the stations for detecting initial and subsequent sample responses of a received substrate; means for monitoring such responses at each of the stations; whereby continuous monitoring is obtained of changes occurring at all of the stations. One of the sensing and monitoring means includes at least one of the following: means for detecting the existence of equilibrium conditions in the substrate, defect test means for determining operability of the substrate for the analyte of choice, and discriminating or testing means for discriminating between a substrate for one analyte and a substrate for a different analyte.

In accordance with another aspect of the invention, the analyzer comprises station means for receiving a substrate capable of generating a response when contacted with analyte-containing sample and means for monitoring responses of the substrate contacted with the sample; the analyzer being improved so that the station includes means for detecting, after receipt of the substrate by the station means, the presence of an inoperable substrate, as herein defined, whereby the inoperable substrate can be discarded without waiting for the completion of the analysis of that substrate.

A preferred use of the invention is simultaneous detection of signals at multiple stations. Therefore, in accordance with another aspect of the invention, there is provided a continuously monitoring, multichannel analyzer for ion activity of sample biological liquids deposited on a substrate containing a pair of ion-selective electrodes, the analyzer comprising a plurality of stations each of which is capable of receiving a substrate for analysis; individual sensing means at each of the stations for detecting initial and subsequent potentiometric signals in the substrate generated by the sample, the sensing means including an electrometer; and monitoring means for monitoring the signal at each of said stations; whereby continuous monitoring is obtained of changes occurring at all of the stations.

The present invention will now be described by way of example with reference to the accompanying drawing in which:—

Figure 1 is a plan of an analyzer constructed in accordance with the present invention;

Figures 2 and 3 are perspective views of one embodiment of an arrangement of stations of the analyzer, only one station being shown for clarity;

Figure 4 is a perspective view of the interior of the station of Fig. 3, the cover plate having been broken away;

Figure 5 is a plan of the interior of the station of Fig. 4;

Figure 6 is an elevation of the pivot member of the station;

Figure 7 is a schematic view of a circuit useful in the station of the present invention;

Figures 8a and 8b are representative plots of the electrometer output and the first derivative of that output, respectively;

Figure 9 is a fragmentary elevation, partly schematic, in section of the stations and the loading and unloading means;

Figure 10 is a fragmentary plan of the transfer means useful in loading the stations;

Figure 11 is a fragmentary elevation in section of the means shown in Fig. 10;

Figure 12A is a fragmentary elevation in section of the metering station, taken generally along line XIIA–XIIA of Fig. 1;

Figure 12B is a fragmentary section taken generally on the line XIIB–XIIB of Fig. 12A;

Figure 13 is an elevation in section of means for supplying the slides to the transfer means, taken generally on line XIII–XIII of Fig. 1;

Figure 14 is a plan of another embodiment of the present invention; and

Figure 15 is a fragmentary elevation in section of one of the stations illustrated in Fig. 14.

Although the present invention is hereinafter described in connection with a multichannel, continuously monitoring potentiometric analyzer for reading ISE's, a preferred embodiment, it is not so limited except where stated. Thus the present invention can also be applied to a continuously monitoring analyzer using multiple radiometric detectors, prefera-

bly either photometers or fluorimeters or both, which read any suitable substrate incorporating, for example, reagents that create a dye in proportion to the analyte being measured, to provide early detection of a defective or erroneous reaction. Such reagents can be in solution or in the form of a dried coating.

As used herein, a "substrate" is the chemical or chemicals through which the analyte of choice of a liquid sample generates a detectable response or signal. That is, a "substrate" is, in the case of colorimetric analysis, one or more reagents, and in the case of potentiometric analysis, one or both electrodes. As used herein, an "inoperable substrate" is one that is either defective or erroneous i.e. unable to measure the analyte for which it has been selected.

In the case of potentiometric analyzers, the substrate which makes the test possible comprises a supported pair of electrodes selective to the ion activity of choice; thus the name ion-selective electrodes or ISE's. Such electrodes, by the use of a salt bridge, permit the generation of an electrical signal, in the presence of the sample test liquid, that is indicative of the test ion activity and thus the concentration, in a manner well known in the art. As used herein, "response" not otherwise limited includes an electrical signal derived from paired electrodes, and also includes any detectable response of the substrate that is indicative of the level of analyte of choice.

Any generally planar form of the ISE's can be used, preferably in pairs to permit a reference sample to be deposited along with the test sample. One convenient form, illustrated as a slide 10, Figs. 1 and 10, is that disclosed in *Research Disclosure*, Vol. 157, May 1977, Publication No. 15767, published by Industrial Opportunities Ltd., Homewell, Havant Hampshire PO91EF United Kingdom. Such a slide comprises, as best seen in Fig. 10, a frame 12 the bottom surface of which is supportingly attached to a pair of ISE's 14 and 16 having a generally planar strip form, and a bridge 18 used to promote ionic migration between the reference sample and the test sample deposited over the electrodes. The bridge is located in one opening 20 of the frame 12, and silver chloride coated surfaces of the ISE's are exposed in two openings 22 of the frame, for purposes of making readings as hereinafter described.

An analyzer constructed in accordance with the present invention can be designed to receive substrates for reading only after the test sample has contacted the substrate, or it can be designed to receive the substrates before that event, to be read "in blank." The former situation, shown in Figs. 1–13, preferably utilizes, in conjunction with an analyzer 30 on a platform 31, means 170 for transferring a substrate in the form of a slide 10 contacted with a sample directly to the analyzer, without passing through intermediate stations. Such an arrangement ensures that the analyzer 30 will sense the initial signal generated by the samples when they first contact the ISE's. The liquid samples can be deposited from containers C on to the ISE slide 10 by hand such as by any suitable disperser or metering slide 10 by hand or by any suitable dispenser or metering station 230, Figs. 1 and 12A. The slides 10 can be loaded on to transfer means 170 by hand or from a suitable supply means 280. Representative, useful mechanisms for station 230 and supply means 280 are discussed hereinafter.

The analyzer 30 comprises a plurality of stations each of which is provided with sensing means for sensing responses or signals from the substrates (Figs. 2–5), computer/-monitoring means 166 to monitor or display the response sensed at each station (Fig. 1), and multiplexing means 160 for repeatedly switching the monitoring means to each of the stations (Fig. 1). As shown in Fig. 2, the stations can be a plurality of substantially identical compartments removably stacked in a frame 42, the frame being movably mounted for vertical displacement with respect to transfer means 170. A convenient mode for such displacement, Fig. 2, comprises mounting the frame 42 in a track 44 mounted on a stanchion 45 supported by a base 46. The back plate 47 of the frame is drilled and threaded, through which is passed a rotatable lead screw 49. The screw is turned by gears 50 and 52 and a motor 53. Each of the stations is preferably separately removably mounted, to facilitate repair or replacement, in frame 42 by opposed tongues 54 which fit into mating grooves 56 of the frame, Fig. 3. A pair of removable, vertically extending stop plates, of which only the front plate 57 is shown, Figs. 3 and 4, prevents the stations from moving out of the grooves 56 during operation.

Preferably each station includes, for sensing initial and subsequent signals of the slide 10 contained therein, contacts in this case in the form of probes 60, one for each ISE, and an electrometer 120 wired to the probes, Figs. 4 and 5. As the stations are identical, one (32) will be described. The location of three of the others is shown in Fig. 3 by dashed arrows labeled 33–35. Station 32 comprises a bottom wall 63, a cover plate 64 which is preferably removably mounted as by screws, front wall 65 and rear wall 66. The probes are disposed to project into a path 62 which the slide 10 follows through the stations, defined by entrance slot 67, bottom wall 63, guide walls 68 upstanding from bottom wall 63, and an exit slot 69, Fig. 3. Adjacent to slot 67, wall 63 is sloped upwardly and inwardly, Fig. 9, for easy loading. Probes 60 are in the form of Z-shaped members with spaced serrations 70 at one end and stop

flanges 71 at the other end (Fig. 6). The Z-shaped members are held in place such as by screws 72 on a pivot member 74. Member 74 in turn comprises a base 76 and a central, upstanding shoulder 78 perforated at front portion 80 and rear portion 82. Base 76 features a pivot lug 84 journaled to guide walls 68 by a pivot pin 86, Fig. 9, at a distance sufficiently spaced above bottom wall 63 as to accommodate slide 10.

To bias pivot member 74 downwardly against a slide in position as shown in Fig. 9, a bias spring 90 is secured at one end to front portion 80 and at its opposite end to a link plate 91 attached to an adjusting screw 92 mounted in front wall 94 of the station. The spring is selected with a spring constant sufficient to rotate member 74 about pivot pin 86 and to force serrations 70 through the non-conductive silver chloride coating that is exposed at opening 20 of the slide and into contact with the conductive silver layer below (Fig. 9). By such means, probes 60 are constantly in contact with the electrodes of slide 10 to provide continuous read-out.

To raise probes 60 during loading and unloading of the slides, a release arm 100 is pinned at end 102 to portion 82 of the pivot member, Figs. 5 and 9. The opposite end 104 of arm 100 projects through opening 106 of rear wall 66, and terminates in handles 108. A solenoid 110 is disposed, Fig. 3, on arm 112 mounted on stanchion 45. The solenoid is provided with jaws 114 which mate with handles 108 to withdraw the arm 100, causing member 74 to pivot away from the slide against the action of spring 90. Stop rods 116 are held in place in rear wall 66, by means such as nuts, to stop the pivoting of member 74 (Fig. 9). Suitable conventional control means, not shown, are wired to the solenoid to activate it on command, and specifically at the time when a slide is to be moved in or out of the station.

The electrometer 120, Figs. 4 and 5, is preferably an FET type and can be disposed outside of guide walls 68 but within the compartment. The electrometer is connected via amplifier 121 and lines 122 to multiplexer 160, hereinafter described. Amplifier 121 can be any conventional type, such as FET (Figs. 5 and 7).

Preferably, each station has its own electrometer because classic conventional multiplexers are unable to respond to low signal levels that are generated by the ISE's prior to the electrometer read-out. However, a single electrometer could be incorporated in monitor/-computer 166 if a mechanical switching unit or some other or no multiplexer is incorporated to handle the signals from each station lacking an electrometer.

Additional circuitry for voltage and impedance test purposes hereinafter described is preferably disposed within each station 32, Fig. 7 (not shown in Figs. 4 and 5), for similar reasons. That is, internal impedances of multiplexers make impedance and voltage comparisons impractical unless they are done prior to multiplexing. However, if such impedances are compensated for by computer logic, even the circuitry hereinafter detailed could be located downstream of multiplexer 160 rather than upstream.

One example of such test circuitry is a calibrating circuit 130 comprising a test voltage source 132, providing a voltage V, wired in series with a load resistor R_1. The calibrating circuit 130 can be closed by a double pole, double throw switch S_1 to the position B–B', Fig. 7. A double pole relay 136 can be used to short out source 132 by closing switch S_2 providing a zero value intercept. The electrometer's reading of a voltage nominally equal to "V" and a shorted voltage E_{short} nominally equal to zero allow the computer, combined with monitor means 166, to calibrate itself. That is, an electrometer reading E_{132} of the nominal voltage V_{132} creates a correction factor $k = V_{132}/E_{132}$. Thereafter, the actual potential $V_{unknown}$ developed by the substrate under test can be determined by the following equation:

$$V_{unknown} = k(E_{unknown} - E_{short})$$

wherein $E_{unknown}$ is the reading of the electrometer for the test in question.

Still further, relay 136 can be oppositely activated to close switch S_3 activating a defect test circuit 140 comprising resistors R_2 and R_3 and switch S_4 in series with probes 60. The function of this circuit is to ascertain in general the operability of the substrates selected for the test. More specifically, in the case of ISE's the circuit is intended to determine the internal impedance of slide 10 and thus whether the coatings of the ISE's of slide 10 are inoperable. This is done with switch S_1 closed to place electrometer 120 in parallel with circuit 140. Switch S_4 is then closed to place R_2 or R_3 in the circuit. R_2 and R_3 become voltage dividers, and the voltages thereacross are in part controlled by the internal impedance of the slide. By selecting the value of R_2 and R_3 to give a predicted ratio of electrometer readings E(open) to E_{R2} or E_{R3} within a fixed value, for example between about 0.5 and 1.5, the internal impedance can be checked. Useful values for the resistors include R_2, for potassium ion determination, equal to above 10 M ohms, and R_3, for chloride ion determination, equal to about 500 K ohms. These values are based on the fact that typical ISE's for potassium ions have impedances Z_s between about 5 megohms and 75 megohms and for chloride ions, Z_s between about 50 K and 130 K ohms.

Conventional comparator circuit components, not shown, can be used to make the

determination whether the slide is chloride ion or potassium ion sensitive, by comparing the ratio of E_{open} and E_{R2} or E_{R3} against a stored constant, and $E_{open}/E_{R2\ or\ 3}$ against the same or different constant, within a suitable range such as the one noted above. Any reading outside of the set range is indicative of a defective coating on one or more of the electrodes, and the slide is discarded. Switch S_4 is of course appropriately selected to R_2 or R_3, depending on which ion is being checked at the time.

If, on the other hand, E_{R2} or E_{R3} is equal to zero initially, and the first derivative of E_{R2} or E_{R3} is approximately equal to zero as explained below, then the paired electrodes of slide 10 are shorted together as a pair and again the slide must be discarded.

By these means, circuit 140 becomes a discriminator circuit which determines, for slides 10 having impedances that are not equal to zero, whether the slide is suitable for measuring chloride ions or whether it is suitable for measuring potassium ions. The impedance value must be comparable to that expected for the desired test, or the computer will register a "failure" due to inoperability, and a new slide 10 is automatically selected for remetering from the sample container still at station 230.

To provide a means of ascertaining equilibrium conditions in the substrate, a circuit to ascertain the rate of change of the signal is included. A preferred form of such a circuit is a conventional differentiator circuit 148, comprising an amplifier 150, such as an FET type, capacitor 152, and resistor R_5 in parallel with the amplifier 150, Fig. 7. Such a circuit preferably is located between multiplexer 160 and monitor/computer 166, as shown, or it can be included as a component of each of stations 32–39. It is useful in ascertaining an equilibration in the initial signal, prior to loss of source of sample at the metering station as hereinafter described. That is, since the voltage values detected by the electrometer with S_1 in the left hand position, Fig. 7, could be caused by a shorted pair or by the condition

$$\text{activity}_{unknown} = \text{activity}_{reference\ fluid,}$$

a derivative not equal to zero will be indicative of the latter and the slide need not be discarded. Furthermore, continuous monitoring of the first derivative allows monitor 166 to determine when equilibration is complete and equilibrium is reached, so that the final reading can be taken. Some slide constructs may require longer equilibration time than others, and circuit 148 ensures that the time at which equilibrium conditions are reached, will be detected for each slide. Thus, each slide will be retained in its station only long enough to obtain a stable reading.

Alternatively, the circuit 148 could be located in monitor/computer 166.

The plots shown in Fig. 8a and 8b illustrate the usefulness of the differentiator circuit. The electrometer reading of zero at time t_1, Fig. 8a, could mean a shorted pair of electrodes. However, circuit 148 detects that the first derivative at time t_1 is not zero, Fig. 8b, so that the short can be ruled out. Such time-varying signals, are to be expected for some initial period of time for the ISE's described. Although the electrometer reading continues to fluctuate until time t_3, the detection of a zero first derivative at time t_3 is indicative that a stable equilibrium condition has been reached and the final reading can be taken.

A conventional second derivative differentiator circuit, not shown, can be added at point A, Fig. 7, to discriminate a momentary $dE/dt = 0$, at time t_2, from the actual equilibrium conditions at time t_3. Alternatively, computer 166 can require more than one first derivative reading to be equal to zero, to distinguish from the nonequilibrium condition existing at time t_2.

Stations 33–39, not shown, are substantially identical to that described for station 32.

It will be readily appreciated that the stations should each provide an electrical shield. Preferably this is achieved by constructing walls 63, 65, 66, cover plate 64, and the exterior side walls from a grounded conductive material, such as copper. The rest of the stations, such as guide walls 68, can be nonconductive materials such as plastic.

To maintain a uniform temperature for the compartments, frame 42 can be cooled by suitable means, for example, passageways 154 which extend above (Fig. 9), below and between the compartments. These carry a liquid for heat exchange. The liquid can be brought in via inlets 156 and removed via outlets 158 (Fig. 3).

Lines 122 extend from each station through a grommet 159 (Fig. 5) and opening on wall 66 and are connected to multiplexer 160, Figs. 2–3, shown as being mounted on frame 42. Alternatively, multiplexer 160 can be fixed to an immovable support, for example, base 46. A line 162 connects from outlet posts 164 of the multiplexer to computer/-monitor 166 (Fig. 1). Any conventional multiplexer can be used, such as the Datel Model MM8 Multiplexer, manufactured by Datel Systems, Inc. Although multiplexing, technically speaking, is well known to be a noncontinuous transmission of individual signals arising from the repeated switching to each of several broadcast sources, such switching occurs at such a rapid rate that, practically speaking, continuous reading of each station 32–39 is achieved. Thus, typical delays between each reading of station 32, for example, are on the order of 1 millisecond, compared to a much slower signal change rate, generated by the substrate, of about 1/6 of a millivolt per

second. The millisecond delay is more than ample to detect such a signal change through the differentiator circuit 148.

Computer/monitor 166 can be of conventional construction, including on its face appropriate dials, gauges or other indicators. 168. A keyboard, not shown, can be added for ease in control. The programming of such a computer can be by means of hardware or by an appropriate program, as is well known. A variety of microprocessors are available in the art for such purposes. Alternatively, only a monitor 166 can be included, the control of the apparatus being provided by a general purpose computer, not shown.

As alternatives to the use of a multiplexer, the signals from each station 32 can be fed directly and continuously (not shown) into a monitoring means comprising a dedicated dial or indicator such as indicator 168, Fig. 1. Or the monitoring means can comprise a single indicator 168 and a manual switch (not shown) which selectively connects in a conventional manner any of the station responses to the indicator for a selective reading of such responses. Such alternatives are more desirable if only a few stations are utilized.

As shown, monitor/computer 166 preferably includes differentiator circuit 148, but not the electrometer or any of the test circuitry illustrated in Fig. 7. However, with appropriate adjustments as mentioned above, it is contemplated that monitor/computer 166 could include such circuitry, so that the sensing means of each station 32–39 includes only the probes 60.

Circuitry similar to that described above can be used in stations 32–39 to detect radiometric signals, except of course without an electrometer 120 and its calibrator circuit 130. In such a case, a photocell detects light reflected from or transmitted through the substrate inserted into path 62, and the differentiator circuit, if it detects a zero first derivative after a suitable initial time increment, will indicate that no reaction is occurring or is occurring at the wrong wavelength. Such results are indicative that the substrate is defective or erroneous and must be discarded without waiting for completion of the test.

As suggested above, it is important that the initial signal generated by the sample on the substrate, whether a measure of a potential or a density change, be detected by the analyzer to determine if the substrate must be discarded and a new one used, or if the substrate is properly functioning and can be retained. By such a procedure, if a new substrate is needed, the same container C of sample can be used to deposit a fresh sample on that new substrate, since that container can be left in the metering position at station 230 (hereinafter described) for the short length of time the initial reading requires. However, to prevent undue delay in the presentation of a new serum sample at station 230, it is preferred that the initial reading be taken as soon as possible. It has been found that a useful time limit is 30 seconds after the sample initially contacts the substrate. This time is sufficient for a computer decision to be made concerning the substrates, and is not long compared to the total processing time. Thereafter, if a "go" signal is generated by the test circuits, the container C from which the sample was taken can be moved out of the metering position at station 230 to a discard station, not shown. To insure that an initial reading is obtained, the substrate with the sample on it is placed within the analyzer directly after the sample is deposited. As used herein, "transferring directly" means transferring without proceeding through an intermediate processing station. Preferably such direct transfer takes place within 30 seconds from the time the sample is deposited on the slide.

Such a direct loading can be done by a transfer means 170, although loading can also be done by hand. Such a means can be constructed, Figs. 10 and 11, to comprise an arm 172 mounted on a rotatable platform 174 fixed to a drive shaft 176 driven by gears 178 and 180, and motor 182. Within arm 172 is reciprocated a pusher element 184 guided between tracks or ways 186 and 188. Each track is appropriately recessed at 190 to accommodate both the pusher element 184 and a slide 10. Springs 192 and 194 serve as a gripping means to temporarily retain the slide in arm 172. To reciprocate element 184 in the tracks, a two-member crank 196 is eccentrically journaled to element 184 at end 198, and at its opposite end 200, to a drive shaft 202 driven by motor 204.

Motor 182 is activated to rotate arm 172 into a position aligned with slot 67, Fig. 9. Then motor 204 is activated so that crank 196 causes pusher element 184 to eject the slide 10 out of tracks 186 and 188 into slot 67.

Transfer means 170 also serves to provide nonsequential access of the substrates to stations 32–39 as soon as computer/monitor 166 identifies a station that can be used. That is, slides can be removed from stations 32–39 by means of incoming new slides pushing out the "finished" slides through slot 69. A conveyor belt 206, shown in phantom, Fig. 9, can be used to remove and discard such "finished" slides. Such use of transfer arm 172 and an incoming slide 10 to eject the old slides depends, as noted, on a computer-generated signal that the old slide has reached equilibrium and that a final reading has been taken. Since such conditions can vary from slide to slide, the vertical movement of frame 42 past transfer arm 172 allows nonsequential selection of the first station

ready to be occupied by a new slide.

Alternatively, a separate unloading mechanism 210 can be utilized at a level 211 which is different from the loading level of arm 172, permitting unloading to occur separately from loading. Such a mechanism can comprise a pusher arm 212 secured to a pin 214 having flanges 216 mounted between tracks 218, of which only one is shown, Fig. 9. Pin 214 is journaled to a crank 220 at end 221, the crank being secured at its other end 222 to a pivot 224. Intermediate the crank ends, the crank accommodates a roller 226 in a slot 228, eccentrically mounted on the rotating face of motor 229.

To deposit a test sample and a reference sample on slide 10 automatically, a metering station 230 is preferably included. Such a metering station includes, Figs. 1 and 12A, a turntable 232 provided around its rim with apertures 234 which accommodate containers C. Such containers conveniently have the form of a cup, and to provide accurate dispensing of stable, pendant drops the cup preferably includes an apertured platform 240. Such as apertured platform can be constructed as described in *Research Disclosure*, Vol. 133, May 1975, Publication No. 13360, published by Industrial Opportunities Ltd., Homewell, Havant, Hampshire, PO9,1EF, UK, so that a fixed, predictable drop volume, such as 10 microlitres. is formed when the liquid in the container is pressurized by means such as hose 242, even when the properties of the liquid vary from patient to patient. Hose 242 is located at a metering frame 244, Fig. 1, which mounts a separate dispenser tube 246, Fig. 12B, for dispensing a similarly sized drop of reference fluid having a known concentration of the ion being analyzed. Because the reference fluid is always the same, it is not necessary that tip 248 of tube 246 be constructed with the same features as platform 240, although it can be.

To permit dispensing when cup 234 is in position in the frame 230, the platform 232 is raised, arrow 247, Fig. 12B, by moving means 249, Fig. 12A, such as a hydraulic piston, to dispose the cup in position under hose 242. At the same time, platform 232 moves past stationary tube 246 by reason of platform aperture 250, Figs. 1 and 12B. The drops shown in Fig. 12B are formed by pressurizing hose 242 and tube 246.

To touch off the pendant drops onto slide 10, arm 172 is raised an appropriate distance, arrows 252, until the slide contacts the drops, but not so far as to contact the slide to tube 246 or platform 240. A suitable mechanism to achieve this action comprises the mounting of shaft 176, Fig. 11, for reciprocal motion in platform 31. Preferably, a collar 254 is fixed to the shaft and an eccentric 256 is coupled to the collar and activated by motor 258 to raise platform 174 and arm 172 at the appropriate time.

The indexing of table 232 is achieved by a motor 260 activated by computer/monitor 166. Preferably, the computer 166 is programmed to delay any advance of motor 260 until the initial response is detected, i.e., the tests described above have indicated that the substrate is operative for the analyte of choice, however long such delay might take.

Alternatively, the advance of the turntable can be delayed 30 seconds by a delay circuit, Fig. 12A, which can comprise a limit switch 262 which alerts computer 166 that dispensing has begun; and a conventional 30-second timer 264 which delas the signal from the computer to motor 260. This permits the initial reading of the substrate as noted above, before the sample container from which the sample was taken is moved away from station 230.

Blank slides 10 can be inserted into arm 172 by hand, or, preferably, by an automatic supply means 280, Fig. 13. Such a mechanism comprises a turntable 282 driven by a rotating shaft 284, gears 286 and 288, and motor 290. The turntable carries a plurality of cartridges 292 removably confined within housings 294 spaced around the circumference of turntable 282. Each cartridge contains a stack of the slides 10 appropriate to the test to be run, the slides of any given cartridge all being specific for the same test. An ejector 300 is mounted below turntable 282, and is constructed similarly to unloading mechanism 210. That is, a pusher arm 312 is connected to a pin 314 which rides in a track 318 as directed by a crank 320 pivoting at 324 and driven by a roller 326 and motor 329. Arm 312 slides into a slot 330 in cartridge 292 to eject a slide 10 out exit slot 332 into the waiting transfer arm 172. Arm 312 is then withdrawn, and either another slide from the same cartridge, or a slide from another cartridge, is pushed out by arm 312 when transfer arm 172 returns to pick up another slide.

As will be apparent from the preceding, the operation of the aforedescribed apparatus is in the reverse order from that in which the stations were described. That is, supply means 280 is first activated to eject a blank slide from its cartridge 292 into transfer arm 172. Arm 172 is rotated counterclockwise, Fig. 1, until the blank slide is positioned under metering frame 244 of metering station 230. At the same time, turntable 232 is rotated until container C of choice is positioned under hose 242, and aperture 250 under tube 246. The pendant drops are formed and arm 172 is raised to touch off the drops. Arm 172 is lowered, and is rotated further until aligned with slot 67 of one of the stations 32–39. During the slide and sample dispensing, analyzer 30 indexes frame 42 up or down screw 49 until the appropriate station is at the level of arm 172 when it arrives with

a new slide bearing deposited test and reference fluids. Motor 204 is activated, and pusher element 184 ejects the slide into the station of the analyzer, preferably within 30 seconds from the time the drops were touched off onto the slide. As the slide advances into the compartments of that station, arm 100 is pulled to raise probes 60, and released when the slide is in the position shown, Fig. 9. The defect test circuit 140 is activated, and the first derivative is considered to be certain the substrate is not defective or erroneous as to type. If the substrate must be replaced, immediately a new slide is dispensed by supply means 280, a sample drop is dispensed onto the new slide at station 230 from the same cup as before, and the old slide is discarded or replaced by the new one. If the substrate is found to be satisfactory, turntable 23 can index to a new cup C or deposit the same sample in the same cup on a different slide, for a different ion concentration. The first derivative continues to be measured until an appropriate zero derivative value is reached, at which time a final reading is taken by the electrometer and the slide can be discarded.

Appropriate control circuitry, not shown, can be used to insure that the aforedescribed machine functions are followed in their necessary order. For example, conventional limit switches can be positioned to detect completion of a movement which is a condition precedent. An optical detecting circuit can be used, including a light source, not shown, on arm 350, Fig. 2, positioned to detect slot 67 and thereby initiate a command that designates that the indexing of frame 42 to the station 32–39 of choice is completed.

Figs. 14 and 15 illustrate an alternate embodiment wherein the substrate is read "in blank" at the several stations of the analyzer before any fluid is deposited by the metering station, as well as afterwards. Parts similar to those previously described bear the same reference numeral to which the distinguishing suffix "a" has been applied.

As in the previous embodiment, slides 10a are dispensed by a supply mechanism 280a comprising a turntable 282a, a plurality of cartridges 292a, and an ejector, not shown. Samples are metered from a metering frame 244a at dispenser station 230a, again using a turntable 232a bearing sample cups C. Unlike the previous embodiment, however, stations 32a, 33a, etc. are disposed on a turntable 400 which replaces the transfer means 170 of the previous embodiment, turntable 400 being the means by which each slide, now in a reading compartment, is moved into position at the metering stations 230a. Each station 32a, etc., such as station 32a, Fig. 15, has all the circuitry, probes 60a and probe release arm 100a as described before, except that in addition an additional switch S_1', shown in phantom, Fig. 7, is needed, along with a dispensing slot 402 formed in compartment cover plate 64a to receive the sample and reference drops. To complete the electrical isolation of compartment 32a, pivoting metal doors 404 mounted on spring-biased rods 406 can be disposed in slot 402 to be pushed out of the way by dispensing tube 246a and the sample cup, not shown. Preferably platform 232a moves up for dispensing in the manner shown for the previous embodiment, Figs. 12A and 12B, as does turntable 400 to cause touch-off of the drops. Since the slides cannot pass completely through turntable 400, discarding is achieved by a pusher element 410 mounted on a track 412 and activated by a crank 414 under the compartment, which ejects the "finished" slide out the entrance slot 67a through which it came.

This arrangement permits the substrate or slide 10 to be tested with no sample present. Such tests include the "short" test obtained by reading the electrometer when S_1 is in position C–C' and S_1' is closed to connect voltage source 132 to the ISE's. If the reading is not equal to the nominal zero value obtained by thereafter closing S_2 to short out voltage source 132, then no short exists. If a short does exist, the slide is immediately discarded in favor of a new one even before the compartment is rotated into position under metering frame 244a, saving sample which is otherwise lost if a defect is discovered after sample has been metered or dispensed.

An incubator 420 can also be provided, Fig. 14, into which the compartments 32a, etc. are rotated. This construction can obviate the need for heating or cooling tubes in the compartment, shown as tubes 154 in Fig. 9.

CLAIMS

1. Apparatus for analysis of liquids of the type which accepts disposable discrete analyte dedicated substrates and monitors the respone of the substrates to applied analyte samples characterised in that testing means associated with the response measuring means includes testing means adapted to discard substrates which are inoperable due to a defect or being dedicated to a different analyte from that being measured.

2. Apparatus as claimed in Claim 1 which includes a plurality of stations, each of which is constructed to receive a substrate bearing a sample for analysis, and each of which is adapted to discard substrates which are inoperable.

3. Apparatus as claimed in Claim 2 which includes means for mounting the substrates one above another in a stack; individual sensing means at each of the stations for sensing initial and subsequent sample responses of a liquid analyte contacted substrates; transfer means for inserting and removing the substrates into and out of any one of the stations in response to the sample responses sensed

by the sensing means; means for moving the stack vertically with respect to the transfer means; and testing means for monitoring the sample responses at the stations; whereby any one of the stations can be unloaded and reloaded immediately on detection of a response by the testing means indicative of an inoperable substrate.

4. Apparatus as claimed in any of the Claims 1 to 3 in which the testing means is capable of detecting a zero rate of change of the sample response of a liquid analyte contacted substrate and initiating its discard.

5. Apparatus as claimed in any of the Claims 1 to 4 in which the response measuring means is a colorimeter or fluorimeter and the substrate responds radiometrically to a liquid analyte sample.

6. Apparatus as claimed in any of the Claims 1 to 4 in which the response measuring means is electrical and measures to potential of the substrate which is a pair of ion selective electrodes.

7. Apparatus as claimed in Claim 6 in which the testing means associated with the response measuring means measures the impedance of the ion selective electrode.

8. Apparatus as claimed in Claim 7 in which the testing means initiates discarding of the substrate if its impedance is substantially zero.

9. Apparatus as claimed in Claim 8 in which the testing means includes a resistor connected in parallel with the ion selective electrode having a resistance value less than the minimum impedance of the ion selective electrodes.

10. Apparatus as claimed in Claim 7 in which the testing means is capable of discriminating between at least two different ion selective electrode impedances.

11. Apparatus as claimed in Claim 10 in which the testing means is capable of discriminating between the impedance of a chloride ion selective electrode and a potassium ion selective electrode.

12. Apparatus as claimed in Claims 10 or 11 in which the testing means includes a resistor having a resistance value between that of the impedance of the two ion selective electrodes.

13. Apparatus as claimed in Claim 1 and as herein particularly described.

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